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POLLEN STORAGE AND VIABILITY

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ABSTRACT

Pollen storage is useful for breeding programmes, genetic conservation, artificial pollination and self-incompatiblility. Longevity of pollen varies greatly with plant species and storage conditions. Many techniques are followed these days to maintain the viability of pollen under storage conditions. Mostly binucleate pollen can he stored for long periods of time without loss of viability as compared to trinucleate pollen. Pollen stored at low temperature presented germination capacity better than high temperature. There are many factors which influence the pollen viability. This review highlights the storage conditions for pollen and factors affecting its viability.

KEYWORDS: Pollen Longevity, Storage, Cryopreservation

INTRODUCTION

Pollen represents a critical stage in the life cycle of plants, as viable pollen is crucial for efficient sexual plant reproduction. The quality of pollen is assesses on the basis of viability and vigour. Viability refers to the ability of the pollen to deliver functional sperm cells to the embryo sac following compatible pollination (Shivana and Ram, 1993). Mature pollen grains are relatively quiescent, and contain small quantities of reserve food, usually in the form of starch or sugars, lipids and proteins.

The cytoplasm of viable and mature pollen is non-vacuolate, granular and contains many plastids, mitochondria, golgi derived vesicles, lipid droplets and a large population of quiescent dictyosomes (Kroh, 1967). The changes which procede and accompany germination are rapid and dramatic. The permeability of the pollen plasmalemma is altered during hydration of pollen at the time of germination (Wang et al., 2004). Water uptake and activation or synthesis of enzymes are the basic initiating factors for germination. Respiration and metabolism of external sucrose by germinating pollen is evident long before tube growth becomes visible. An initial high rate of respiration coincides with rapid starch accumulation in germinating pollen (Dickinson, 1967; Rounds et al., 2011). During the activation phase, before the emergence of pollen tube, protein and nucleic acid synthesis takes place (Young & Stanley, 1963, Molly & Wilson, 1969, Ischebeck et al., 2014).

Many enzymes are present in pollen grain wall and these readily diffuse out into the surrounding medium before the pollen tubes are formed (Stanley and Linskens, 1964, 1965; Kamboj etal 1984). Many of these are hydrolyzing and cell wall softening enzymes (Anderson et al., 2002). Free amino acids are released rapidly when pollen grains are placed in a germination medium (Linskens and Schrauwen, 1969).

Pollen grains of a wide variety of species germinate successfully in sugar solutions. Sucrose is probably the best and most commonly used source of carbon and energy for pollen. It provides and maintains a proper osmotic environment for germination of pollen and continued growth of pollen tubes. Supplementing the medium with boron stimulates

germination and pollen tube growth. Boron is an essential element for plant growth and must be present in adequate amounts to ensure optimal plant growth and productivity. Boron plays a role in flowering and fruiting process of plants and its deficiency results in low pollen viability, poor pollen germination and reduced pollen tube growth (Brown et al., 1994). Boron takes part in pollen germination and style tube formation and therefore has a vital function in fertilization of flowering crops. Boron regulates the hydration of colloids, is associated with polyhydroxyl compounds of the pollen membrane, and is involved in the synthesis of pectic substances for the tube wall (Wang et al., 2003; Acar et al., 2010). Boron added in the form of boric acid, is also essential for the *in vitro* culturing of pollen from most species; for example, it is well appreciated that elimination of boric acid from the culture medium often leads to tube bursting (Holdaway-Clarke and Hepler, 2003). According to Acar (2004), the optimum *in vitro* pollen germination conditions for pistachio trees were 20% (w/v) sucrose medium, hanging drop germination method and 25°C temperature in dark conditions for 24 h. In nature, water, sugar and amino acids are supplied by the style to nourish the growing pollen tube. For many species, boron and calcium are also required for pollen tube growth. Boron, which is provided by stigmas and styles, facilitates sugar uptake and has a role in pectin production in the pollen tube (Richards, 1986).

Pollen grains of many species are known to contain relatively large amounts of plant growth substances, particularly auxins, gibberellins and cytokinins (Malik and Ahluwalia, 1985). It is not surprising that additional amounts of plant growth substances supplied exogenously to pollen fail to produce any marked improvement in pollen germination and tube growth (Johri and Vasil, 1961). For example, GAs also, are present in developing pollen after anthesis and numerous studies have reported the effect of GA application on pollen tube growth *in vivo* or *in vitro* (Singh et al., 2002). Depending on the species examined and the concentration used, GAs can promote, inhibit, or have no effect on pollen germination and tube elongation *in vitro* (Bhandal and Malik, 1979; Viti et al., 1990; Setia et al., 1994).

The pollen tube grows as a result of stretching of the wall caused by various factors including turgor pressure. Such a stretching is restricted to the tip region where new material is being deposited *via* fusion of vesicles with the plasmalemma. Kroeger et al., 2009 suggest that vesicles move forward along the cortex by the actin fringe and are released into the inverted cone. From this point, vesicle motion may be controlled by diffusion. Exocytosis provides a path that pulls vesicles acropetally toward the apical plasma membrane (Kroeger and Geitmann, 2012). The non-vacuolated and agranular cytoplasm of the cap block is devoid of cell organelles like mitochondria, golgi bodies, ER, amyloplasts and lipid bodies and shows no cytoplasmic streaming in growing pollen tubes.

The activation of the relatively quiescent pollen grain during germination is accompanied by protein synthesis, which is followed by RNA synthesis. The greatest concentration of RNA in pollen tubes is found in the growing tip region, which lacks ribosomes but is packed with smooth membranes that are sensitive to RNase treatment. Proteins synthesized during germination of pollen are required for germination and early pollen tube growth (Hao et al., 2005). Actinomycin D and cycloheximide have been useful tools in the study of pollen tube development (Mascarenhas, 1975, 1993; Raghavan, 1981; Fernando et al., 2001; Song et al., 2002; Yang et al., 2003). Roberts et al. (1984) noticed no significant effect of cycloheximide on pollen germination and initial tube growth in *in vitro*. While, Speranza et al. (2001) reported that kiwifruit (*Actinidia deliciosa* var. deliciosa) pollen germination was completely inhibited by cycloheximide. Fernando etal. (2001) found that the RNA and protein synthesis during pollen germination and tube elongation in coniferous species were inhibited by actinomycin and cycloheximide, respectively.

The present unit reviews on the more recent information on the causes accounting for the loss of pollen viability and on the conditions for prolonging the viability and storage periods.

STORAGE OF POLLEN

Horticulturists and plant breeders have long been interested in crossing varieties, species and even genera to produce new and improved types of plants better suited to human requirements. However, many of these attempts have failed, due to the barriers to crossability, e.g., flowering of the selected parents at different times or at different places, failure of the pollen grains to germinate on the stigma, bursting of pollen tubes in the style, failure of tubes to grow through the style, and slow growth of the tubes so that they do not reach the ovules before abscission of the flower. Occasionally the pollen tubes no doubt enter the embryo sac, but sterility results either from failure of the male gamete to fuse with the egg nucleus or from subsequent failure or arrested development of the embryo and the endosperm (Maheshwari, 1950). The most efficient technique to overcome this barrier, imposed by time and space, is the use of stored pollen. Pollen storage also eliminate the need to continuously grow lines frequently used in crosses. It also provides greater flexibility in experimental studies on pollen.

Short-Term Storage in Organic Solvents

Storage of pollen solvents avoids the problem of maintenance of relative humidity and may be a useful technique for transporting pollen refrigeration. The impoertance of organic solvents in pollen preservation has been initially done by Iwanami (1972 a); further, the relative performance of a number of organic solvents in *Camellia* (Iwanami, 1972 b) have thrown an insight into further studies on other species (Iwanami and Nakamura, 1972). The efficacy of theses solvents for storing pollen of some leguminous taxa has been systematically studied (Mishra and Shivanna, 1982) indicating the species specific responses. The effects of these solvents on leaching of phospholipids (Jain and Shivanna, 1988 a) and their relation to viability resulting through their implications on membrane integrity (Jain and Shivanna, 1988 b) have been further thoroughly reviewed by Jain and Shivanna, 1990. All such studies further point to the differential action of polar and non-polar solvents in their preservation ability; it was claimed that polar solvents like hexane and diethyl ether cause very little leaching of substrates like sugars, phospholipids and amino acids as compared to pollen stored in non-polar solvents. The studies on the moisture content and the dehydration and rehydration effects on pollen indicate that many of the structural and functional changes in the cell are quite irreversible; as such the pollen viability of many taxa could be prolonged for shorter durations through appropriate manipulations of water content and humidity of the storage conditions. The relative roles of moisture and storage temperatures on the period of storage are highly variable for a number of plants (Grewal, 1988).

In many of the taxa of graminae with tri-nucleate pollen, the viability is reported to be very short under natural conditions (Barnabas, 1982). Even the short-term storage conditions are not as effective as in other angiosperm families. It was shown that the wheat pollen with viability up to 1- 3 h at 20°C storage may retain it up to one day at 4°C (Barnabas, 1982). In maize, Jones and Newell (1948) estimated pollen viability up to 9 to 11 days at 4°C and 90% relative humidity; however, this has reduced to six days with a further increase of relative humidity by 5-10% in the studies of others (Pfahler and Linskens, 1973). In *Secale cereale*, the 12 h storage period at 17-21°C storage temperature (Pfundt, 1910) could be enhanced upto 4 to 7 days by lowering the temperature to 4°C at similar humidity levels (Patil and Gaud, 1980; Barnabas, 1982).

In *Pennisetum*, on the other hand, the viability up to 14 days at 14°C (Pokhriyal and Mangth, 1979) was reduced to 4 days at 4°C (Cooper and Burton, 1965). Moreover, the seed set was only 8% as good as fresh pollen when applied after 3 weeks. Pollen studies on pearl millet from our laboratory have shown that the viability of pollen stored either at O°C or at 4°C could not be improved beyond 72h (Raju, 1983). However, studies have shown that storing at 4°C in a dessicator extends the viability in the *Papaver* up to 200 days (Dhingra and Verghese, 1990). The "pollen-dryer" (Barnabas, 1994) seem to be highly effective in the regulation of moisture and relative humidity of air for achieving rapid and uniform dehydration and in the control of temperature and RH in short-term storage methods. Using paramagnetic resonance spectroscopy Buitink et al. (1998) have found that molecular mobility is inversely correlated with storage stability and the mobility is controlled by water content. Such a correlation suggests that storage stability must be at least partially controlled by molecular mobility and these studies provide estimates for optimum storage conditions. However, the information obtained using techniques like spin probe, electron paramagnetic resonance (EPR) and saturation transfer paramagnetic resonance (ST-EPR) by Cheng (2005) and Pacini et al. (2006) have shown that partially hydrated pollen is commonly devoid of mechanisms to conserve pollen viability suggesting that optimum levels of moisture and humidity have to be assessed properly for each taxon.

Long - Term Storage

For a limited number of taxa, the storage of pollen above O°C, although enhanced the period of longevity, the results are not always encouraging; the success is much more limited in graminaceous members. In addition, the application of freezing and cryogenic conditions has become a successful event in the subsequent studies. In spite of quite promising results with these approaches, the outcome of the investigations seem to be influenced by more variables in the protocols under use; these are complicated to a certain extent exhibiting varied responses and are discussed here under.

Use of Freezing Temperatures

Under this category of preservation, long-term storage could be achieved through exposure of pollen to temperatures between -10 to 34°C. The vast amount of information from a large numbers of taxa indicate that the method is more suited for improving the longevity of bi-nucleate pollen with low water content rather than trinucleate ones (Towill, 1985; Barnabas and Kovacs, 1997). Pollen viability of most of the species was enhanced to significantly longer periods with a range of a few months to as high as three years in some cases. The recent studies (Tyagi and Hymowitz, 2003) have recorded that pollen of annual soybean retain their viability for four months when exposed to - 20°C; however, the germination *in vitro* was a failure with the wild relatives as against near normal germination of the stored pollen of cultivars. Similar studies in lily (Wang et al., 2004) resulted in a delay in germination by one hour, in case of stored pollen (at - 20°C) as compared to fresh pollen; degradation of some proteins during storage was presumed and will be synthesized during the lag period and was confirmed in their studies on the qualitative and quantitative differences in the proteins of fresh and stored pollen (Wang et al., 2004).

Freeze-Drying and Vacuum-Drying

This kind of approach of pollen preservation is also referred to as lyophilization. Since the earlier reports of applications of freezing and drying (Harris, 1954), it has become a potential tool for safe storage of a number of biological materials (Sharp and Smith, 1957; Cook et al., 1963). It has also been extended for preservation of pollen of many species to satisfactory levels. In freeze-drying method, the pollen would be initially exposed to rapid freezing temperatures

(-60 to - 80°C) followed by gradual removal of water under vacuum sublimation. In the vacuum-drying, on the other hand, the pollen is directly exposed to vacuum and simultaneous cooling while the moisture is withdrawn by evaporative cooling and does not include the initial freezing step of freeze-drying method.

King (1960) reviewed the usefulness of this method in many of the taxa although the first reports on lyophilization dates back to early decades of 20th century (Knowlton, 1922). The method is further influenced by the conditions of the temperature, humidity and pressure (Snope and Ellison, 1963) as well as the agents used for attaining pressure. The presence of inert gases (like helium and nitrogen) or simply vacuum in the pollen atmosphere profoundly influence the duration of storage (Snope and Ellison, 1963). Hanson (1961) recorded an improvement in the longevity of both pollen and ovaries of alfalfa and later for pollen many other species (King, 1961). The results of vacuum-drying and freeze-drying on the viability of pea pollen are very encouraging (Layne and Hagedorn, 1963). Freeze-drying of pollen for various periods had not changed the seed set, germinability and the seedling vigour of Douglas-fir pollen indicating its potential for safe and prolonged storage (Livingston et al.,1962). Since water molecules sublime with minimum change of molecular configuration in this method, the influence of pre-freezing as well as freezing temperatures of various periods need to be established for improving the storage conditions of pollen for each taxon. Cold storage of pollen prior to freeze drying improved viability in both the undried and air-dried samples in *Pinus monticola* (Ching and Ching, 1964). The rate of removal of water appears to be a function of water content of the pollen and was slower for cold-stored pollen. Ching and Ching (1964) further claimed that air-drying of pollen for 4 hours or slight refrigeration of the air-dried pollen for several weeks before freeze-drying for 30-60 minutes was sufficient to remove free water and to retain higher viability.

The impact of temperature, humidity and pressure on storage ability has also been emphasized while studying the potential of freeze drying on the pollen of *Lilium* and maize (Nath and Anderson, 1975). Barnabas and Kovacs (1997) indicate that such methods are more effective for taxa with desiccation - tolerant pollen to achieve fairly longer periods of viability.

Cryopreservation

The fact that pollen could be preserved in unaltered conditions by exposure to extremely low temperatures (-70 to - *196°C)* has become evident when the method has been first used for the pollen of *Antirhinum majus* (Knowlton, 1922). The potential of the technique is evident from the quite many applications of it in the preservation of pollen of diverse taxa since 1950. The results of utility of cryopreservation of pollen from agronomic species using liquid nitrogen (Collins et al., 1973) has followed further substantiation in a number of species (Frank et al., 1982; Crips and Grout, 1984; Filipova, 1985; Ganeshan, 1986), including many crop species of the family graminae (Barnabas *et al.* 1992). Their implications on storage and germinability (Visser, 1955) and on the pollen of *Pyrus malus* and *Pyrus communis* (Griggs et al., 1950) indicated the practical utility of it. In these plants the viability was extended upto 1 to 2 years whereas the pollen remain viable for as many as 1062 days in the case of *Lycopersican esculentum* by exposing pollen to -190°C (Visser, 1955) and up to as many as 5 Years in *Vitis vinifera* (Ganeshan and Alexander, 1988).

However, the method may not be equally effective for all species. There are reports of preservation for only shorter periods as in *Glycine max* (21 days) and only 10 days in *Gossypium hirsutum* (Collins et al., *1973)* and to about one month in *Vicia faba* (Telaye et al., 1990). In *Solanum tuberosum*, the exposure to -196°C led to the viability of stored pollen from 9 months (Weatherhead et al., 1978) to 24 months (Towill, 1984). In *Capsicum*, cryogenic method has not only extended the longevity (Alexander et al., 1991) but also the stored pollen retained their fertilization ability; similarly

the germination abilities of cryopreserved pollen were on par with fresh pollen in wheat (Andraeica et al., 1988). Ichikawa et al. (1970) and Ichikawa and Shidei (1972) many tree species including larch *(Larix Ieptolepis)* subjecting their pollen to super low temperatures followed by the studies on pollen of other tree plants like peach and pear (Jiang and Gao, 1989). This method have proved equally effective in the preservatioin of pollen of maize (Barnabas and Rajki, 1976) and of horticultural species such as roses (Marchant et al., 1993). Shivanna and Johri (1985) have rightly stressed the need for such methods in their book on cryopreservation methods. Many studies, in the past, indicate the need for reducing the water content below a threshold level before the pollen is exposed to such low temperatures to achieve optimal results (Ching and Slabaugh, 1966; Barnabas and Rajki, 1976; Kerhoas et al., 1987). It is because the dehydrated pollen possess less freezable water and could survive during further exposure to freezing temperatures and point to the need for assessing the critical moisture level for pollen of each species for a successful long-term cryopreservation of the various taxa (Conor and Towill, 1993).

The experiments on germination and tube growth of stored pollen (Patil and Goud, 1980) and their further effects on fertilization and seed set (Pokhriyal and Mangth, 1979) after long-term storage using cryogenic methods indicate insignificant deviations from the normal pollen. The staining protocols like enzymatic fluorescence (Heslop-Harrison & Heslop-Harrison, 1970) are readily useful for quickly assessing pollen viability whereas the fluorochromatic (FeR) technique to determine the quality of the stored pollen (Heslop-Harrison et al., 1984) as has been further refined recently (Shivanna and Rangasamy, 1992) has much potential to assess the further quality of the pollen stored using all these different approaches.

POLLEN BANKS

One major setback in plant breeding is the asynchrony of flowering among the cultivars and their wild relatives hindering the further cross breeding (Vithanage & Alexander, 1985). In addition, many of the recent haploid breeding procedures and biotechnology experiments use the pollen as source material for achieving gene expression of the transformed pollen cells suggesting the need for conservation of pollen; the necessity of "pollen banks" has become a potential approach of conservation of genetic resources analogous with the preservation of sperms and embryos in the case of animals in veterinary sciences which facilitates their further transport to different regions also.

The significance of it was foreseen even in the late 19th century as evident from the words of King (1885) - "control over the supply of pollen, so that we might use it when and where convenient to ourselves". Thus they provide the opportunity for the scientists and breeders to use the source materials irrespective of time and space; further they eliminate the need for using nurseries and green houses for plant growth (Barnabas and Kovacs, 1997).

Moreover, the relatively simpler methods of their collection and preservation and the availability in large numbers in relatively pure form provides the opportunity for their use in gene manipulation studies. Added to these, there is a stable expression of many proteins indicating their further utility in transgenic studies of all higher plants. Keeping in view of all these facts the future studies may be focused on further understanding the mechanisms of damage to the pollen membranes and of maintaining the structural integrity of pollen as related to its longevity and improvement of storage methods to achieve the full potential of the various available techniques of pollen preservation.

FACTORS AFFECTING VIABILITY

Pollen viability after is influenced by a number of biotic and abiotic factors. The longevity of pollen, as influenced

by the bi-nucleate or trinucleate conditions at the time of dispersal (Hoekstra, 1979) was attributed to various structural and physiological changes; further the low moisture content, more resistant cell wall and other features of the two-celled pollen reduce the loss of viability which naturally have a higher life-span as compared to the tri-nucleate pollen (Johri & Shivanna, 1977). Pollen membrane is highly dynamic and labile (Kavanu, 1963), Recent studies on B-chromosomes, knobbed chromosomes and NOR in maize have established that chromosomal instability in the tapetal cells results in programmed cell death (Gonzalez-Sanchez et al., 2004) which further hinders the development of microspores to form viable pollen grains. The relation of the vegetative cell membrane to the viability was also understood to a considerable extent (Shivanna & Heslop-Harrison, 1981). A direct role of membrane components of phosopholipids on pollen viability indicates the occurrence of structural changes in the pollen as a consequence of loss of viability. Further, the actin cytoskeletal elements are likely to be disrupted during water loss from the pollen (Heslop-Harrison & Heslop-Harrison, 1992). The fourier transform infra red spectroscopy (FTIR) studies of pollen (Crowe et al., 1989 a and b) are highly helpful to reveal the changes in the molecular structures (if the membranes; it is claimed that this phase transition accounts for the imbibitional damage in dry pollen which could be addressed through gradual hydration of pollen or by raising temperature to effect transition to the more favourable liquid crystalline phase. All such studies point to the need for developing the strategies that maintain proper integrity of the membrane components in enhancing the pollen viability.

Effect of Humidity

The response to high or low humidity differ between species and is usually associated with the intrinsic hydration state of the pollen at dehiscence (Nepi et al., 2001). Pollen of most species contains very little water but some pollen grains have relatively high hydration levels (Stanley and Linskens, 1974). For example, pollen of the Gramineae contain more than 30% water at dehiscence (Franchi et al., 2002). Besides having higher hydration levels, these pollen grains are also metabolically more active, which allows fast pollen tube extrusion (Heslop-Harrison, 2000). In desiccation intolerant species loss of water is thought to lead to irreversible changes in the pollen membranes (Shivanna & Heslop-Harrison, 1981). To minimize water loss to the environment, pollen grains of various species have developed adaptations. These may involve structural adaptation in the pollen wall or the presence of sucrose in the pollen cytoplasm to reduce water loss and protect membranes (Heslop-Harrison, 2000).

When maize pollen grains exposed to dehydrating conditions of the atmosphere, they lose virtually all viability within approximately 3 hrs, as measured by *in vitro* germination of the treated pollen grains (Luna et al., 2001; Aylor, 2004). Exposure to the environment was associated with a progressive loss of water from the pollen, and germination was severely inhibited at water content values of 30% and lower (Aylor, 2003). During the experiment, the maize pollen grains turned from spherical and white to collapsed and yellow, reflecting the progression of the dehydration process (Luna et al., 2001; Aylor, 2003).

The differences between species in viability loss during exposure to dehydrating environments has been suggested to be related to the type of carbohydrates that are stored in the cytoplasm (Pacini, 1996). For example, maize only contains 5% sucrose and loses viability rapidly through dehydration, whereas *Pennisetum typhoides* contain 14% sucrose and survives much longer (Hoekstra et al., 1989). In most species, it is the absence of water that causes loss of pollen viability, but in some cases also the presence of water in the form of rain can be detrimental for pollen.

For cotton, for example, it has been shown that water causes pollen to burst (Burke, 2002). This phenomenon not only has strong effects on boll set, but also provides opportunities to induce male sterility by applying water to flowering plants.

Pfundt (1910) investigated the effect of 0, 30, 60 and 90 per cent relative humidity (RH) on the viability of the pollen of 140 species at 17-22^{o}C. From the observations it is evident that the maximum longevity was obtained at low relative humidities (0-30%). Manaresi (1924) observed longevity of about one year in apple, pear, grape and plum. King (1965) listed the details of pollen storage of a number of taxa under controlled temperature and humidity. In contrast to the relatively long viability in pollen of other plants, the pollen of the gramineae is extremely short-lived and the range of humidity (0-40%), which is favourable to most other pollens is decidedly harmful. According to Pfeiffer (1936) there is an intimate correlation between longevity of pollen and moisture content of the air, with maxima and minima of different pollens at different humidities.

Effect of Temperature

Mature pollen grains are generally rather resistant to temperature stress applied after dehiscence. In tomato, applying mild heat stress after dehiscence did reduce fruit set, but the differences with controls were not significant (Sato etal., 2002). Experiments in Brassica pollen revealed germination still occured after exposure to 60°C for 4 hrs; if the pollen was prehydrated in humid air prior to germination they even germinated after 24 hrs at 45°C (Rao et al., 1992). However, germination rates and pollen tube lengths were significantly lower than for controls. Seed set after pollination with pollen treated at 75°C or at 60°C for 24 hrs was reduced, but pollination with any of the other samples led to normal seed set (Rao et al., 1992).

Both cold and heat during pollen development can negatively affect pollen viability, depending on the species. In the case of mango trees, for example, cold periods are associated with reductions in fruit set and it was demonstrated that night temperatures below 10°C decreased pollen viability to 50% of controls (Issarakraisila and Considine, 1994). Temperatures between 20-25°C were reported to be optimal for tomato fruit set (Sato et al., 2002). Surprisingly, raising the temperature to 29°C drastically reduced the number of fruits formed and seeds set. Estimation of starch and soluble sugars in tomato anthers and pollen exposed to mild heat stress showed that stressed pollen grains did not display the temporal increase in starch concentrations (Pressman et al., 2002).

In *Arachis hypogaea*, short periods of heat stress during pollen development resulted in drop of fruit set, pollen production and pollen viability. When a heat-sensitive and a heat-tolerant genotype of *Phaseolus vulgaris* were subjected to mild heat stress during development, pollen viability of the heat-sensitive genotype declined from 80% to below 10% after 10 days of heat treatment. The heat-tolerant genotype, however, still produced 60% viable pollen even after 24 days of stress (Porch and Jahn, 2001).

UV-B Radiation

Studies on the effect of UV-B radiation on plant growth and development have mostly been performed in the past 25 years, when it became clear that atmospheric ozone reduction could lead to an increase in UV-B radiation (Caldwell etal., 1998). UV-B radiation (280-320 nm) is normally present in the sunlight that reaches the earth's surface, and it is therefore likely that mechanisms are present in plants to protect them against the damaging effects of UV-B. However, with increased radiation due to decreased ozone these mechanisms could prove insufficient.

On a cellular level, UV-B induced damage may occur in DNA, proteins and lipids. DNA damage can be repaired, and affected proteins and lipids replaced to a certain degree. In addition, some transport processes are perturbed when membranes are targeted by UV radiation, which in turn may disturb cellular processes (Murphy, 1983). On the whole-plant level increased UV-B radiation may result in reduced growth, but responses differ between species and even between varieties (Caldwell et al., 1998). With respect to reproduction, UV-B radiation may alter timing of flowering and the number of flowers formed. The potential damage by UV-B radiation to the pollen during the transfer from the anther to the stigma may be reduced by flavonoids present in the pollen wall. In addition, dispersal of pollen clustered in some way may prevent the inner pollen grains from radiation damage (Dafni and Firmage, 2000).

Physiological and Biochemical Changes

Besides the state of hydration in the pollen, several of the substrates present within the pollen and their further metabolism are also the important factors involved in the loss of viability in some species. Nielsen (1956) recorded the influence of vitamins and their changes contributing to the viability. The reviews of Johri and Vasil (1961) simply indicate the physiological manipulations in the pollen that account for viability. Buitink et al. (1996) have shown that the water content in the pollen controls viability. However, the physical behaviour of water in the desiccation-sensitive and resistant pollen is not much variable suggesting a difference in the physiological functions of the water within the pollen in these two types. Using the novel technique deviced for studying anhydrobiosis, Wilson et al. (1979) recorded reduced rate of metabolism in the dry pollen.

However, the differential viability in many of the plant species was attributed to the deficiency of the metabolites rather than the decreased rates of metabolism (Barnabas and Kovacs, 1997). The physiological role in the loss of viability was also reflected from the changes in the endogenous levels of carbohydrates and organic acids (Stanley and Poostachi, 1962) and has been further reviewed in their book (Stanley and Linskens, 1974). The importance of carbohydrate metabolism and a separate pathway operating in the pollen/microspores was also thoroughly investigated recently (Castro and Clement, 2006); glucose and fructose amounts decrease progressively from anther wall to fluid and from fluid to pollen while any change in the pollen that obstructs this kind of flow into the pollen makes it inviable. Rao et al. (1992) recorded the impact of high temperature stress on viability in *Brassica* species while the heat stress during maturation of pollen in the anthers alter the carbohydrate metabolism and reduce the sugar concentration in the pollen with an accumulation in the locular fluid resulting in a decrease in pollen viability in tomato (Pressman et al., 2002). The histochemical staining of Wang et al. *(2004)* is highly useful, in this context, to study rapidly and precisely the starch and lipid contents. Deficiency of respiratory substrates are more likely contribute to the loss of viability in the tri-nucleate pollen while increased utilization of respiratory substrates accounts for greater viability in the binucleate pollen (Hoekstra and Bruinsma, 1975; 1980) rather than deficiency.

Chilling temperatures with their usual positive effects on pollen storage, may also result in the damage of pollen (Hoekstra, 1984). However, the significant levels of these endogenous respiratory substrates in the pollen of grass species (especially starch) might indicate the inability, but not the deficiency, in their utilization pointing to the possible inactivation of certain of enzymes of degradation (Barnabas and Kovacs, 1997). In fact, this kind of decreased activity of enzymes like amylases and phosphatases was recorded as early as in 1951 (Hoeckel, 1951). Further, King (1960) has shown that the rate of peroxidase reaction is likely to act as an indicator of pollen viability. The more recent molecular approaches involving understanding the structural rearrangements of mitochondrial genome (Sivaramakrishnan et al.,

1991; Smith and Chowdhury, 1991; Sujatha et al., 1994; Wang and Song, 2005; Kim et al., 2006 and Ashutosh et al., 2006) and of the functioning of some mitochondrial enzymes (Yui et al., 2003; Fan et al., 2004; Kirch et al., 2004; Duroc et al., *2005,* Wang et al., 2006) have substantiated the direct role of them on pollen sterility. It is essential that efforts are to be made in future to relate these aspects to viability as well as storage conditions of pollen to achieve optimum responses in such investigations.

CONCLUSIONS

All methods described provides the evidence of utilization of pollen in gene manipulation studies. Future studies may be focused on further understanding the mechanisms of damage to the pollen membranes and of maintaining the structural integrity of pollen as related to its longevity and improvement of storage methods to achieve the full potential of the various available techniques of pollen preservation**.**

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